KINETIC AND THERMODYNAMIC STUDIES OF THE LIGAND SUBSTITUTION REACTIONS OF THE COBALAMINS

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DECLARATION

I declare that this is my own unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

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31 March 2005

ABSTRACT

The ligand substitution reactions of aquacobalamin are fast and hence the usual inertness of the d^6 Co(III) ion has been modified. It is well established that the reactions proceed through a dissociative interchange mechanism; however, previous ligand studies were performed in a KCl medium, which led to the formation of the more substitution-inert chloro complex. The kinetics of aquacobalamin were reinvestigated with the ligands N_3^-, NO_2^-, SCN^-, $S_2O_3^{2-}$, OCN^- and $SeCN^-$ in a $NaNO_3$ medium. The reactions proceeded too rapidly for saturation kinetics to be observed and hence only the second-order rate constants could be obtained. These were corrected for pH and determined as a function of temperature, from which the activation parameters were determined. The donor atom of the ambidentate ligands were investigated and correlations were found between the Mulliken population on the donor atom, the energy of the highest occupied molecular orbital (HOMO) with σ symmetry, and $\Delta H^{\ddagger}_{k_{II}}$, the enthalpy of activation, and $\Delta S^{\ddagger}_{k_{II}}$, the entropy of activation, respectively. Good correlations occurred when the donor atoms were taken to be N for SCN^- and NO_2^-; S for $S_2O_3^{2-}$; O for OCN^- and Se for $SeCN^-$.

The effect that changing the environment of aquacobalamin has on its kinetics was observed by determining the rate constants for the reaction of pyridine with aquacobalamin in water and 70% ethanol. The rates were faster in water and the activation parameters obtained for the reaction of aquacobalamin with pyridine in 70% ethanol are larger than they are for the reaction in water. The larger $\Delta H^{\ddagger}$ arises due to less bond formation between pyridine and Co in the transition state and $\Delta S^{\ddagger}$ is larger because it is dominated by the freeing of the coordinated water i.e. bond breaking is the dominant process in the transition state.

The effects of a bulkier ligand than water on the kinetics of aquacobalamin were investigated. The temperature dependence of the kinetics of the substitution of I^- in iodocobalamin by imidazole, N_3^- and $S_2O_3^{2-}$ was studied. Despite the increase in size of the departing ligand there is still nucleophilic participation of the

incoming ligand in the transition state and hence the reaction still proceeds via an I_d mechanism.

In order to probe the *cis*-effect of the corrin in vitamin B_{12} derivatives, comparative studies were undertaken of the reactions of aquacobalamin and aqua-10-Xcobalamin, X = Cl, NO, NH_2, where the H at C10 was replaced with an electron-donating (Cl, NH_2) or electron-withdrawing (NO) group. Formation constants were obtained for aquacobalamin and aqua-10-chlorocobalamin for the substitution of coordinated H_2O with various anions (N_3^-, NO_2^-, SCN^-, $S_2O_3^{2-}$, OCN^-, $SeCN^-$) and neutral *N*-donor ligands (CH_3NH_3, pyridine, imidazole). The anionic ligands bind more strongly to aqua-10-chlorocobalamin than to aquacobalamin with log *K* values larger by between 0.10 and 0.63 (average 0.26) larger. The converse is true for the neutral *N*-donor ligands, where log *K* is smaller by between 0.17 and 0.3 (average 0.25). Semi-empirical molecular orbital (SEMO) calculations using the ZINDO/1 model on the hydroxo complexes show that charge density is delocalised from the axial donor atom to the metal and Cl. Thus the anionic ligands bind more strongly to aqua-10-chlorocobalamin because of the ability of the metal and the Cl at C10 to accept charge density from the ligand. The cobalt ion in aqua-10-chlorocobalamin is more electron rich than it is in aquacobalamin and so it is less likely to accept further electron density from a neutral axial donor ligand. This results in the stability being lower than that of aquacobalamin.

The reaction kinetics of the substitution of H_2O in aqua-10-chlorocobalamin were determined for the ligands N_3^- and pyridine. The reaction proceeds via a dissociative interchange mechanism since saturation was seen for pyridine and not for N_3^-. The activation parameters, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, are lower for aqua-10-chlorocobalamin than aquacobalamin and hence it can be deduced that bond breaking between the coordinated water and the cobalt atom is more dominant in aquacobalamin. The rates of reaction are faster for aquacobalamin than they are for aqua-10-chlorocobalamin. SEMO calculations show that as the Co–O bond is stretched, the charge density on Co in aquacobalamin is always lower than that on

aqua-10-chlorocobalamin, suggesting that aquacobalamin is a better electrophile towards the incoming ligand, thereby explaining the faster kinetics.

Aqua-10-nitrosocobalamin was synthesised and characterised by FAB(MS), NMR and UV-vis spectroscopy. The strongly electron-withdrawing NO group has deactivated the metal ion towards ligand substitution, with neither 1.2 M pyridine nor 0.7 M N_3^- showing any spectroscopic evidence for the displacement of the axial H_2O ligand. This provides further evidence that the electronic structure of the corrin ring can directly influence the ligand-binding properties of the metal.

Aqua-10-aminocobalamin was synthesised from aqua-10-nitrosocobalamin but is unstable in solution. Hence, only a preliminary UV-vis study could be undertaken with the compound. This study shows that the shifts in the bands occur towards longer wavelengths than that of aqua-10-chlorocobalamin, suggesting that the amino group at the C10 position donates more electron density to the cobalt centre than the chloro group.

This work is dedicated to my Family:

My Parents: John and Deneen Knapton
Siblings: Cindy and Peter
Husband and soulmate: Mark

Dada and Mama – I wish you could have been here to see the end product.

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CONTENTS

Contents *(continued)*

Contents *(continued)*

Contents *(continued)*

Contents *(continued)*

Contents *(continued)*

Contents *(continued)*

LIST OF FIGURES

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

List of Figures *(continued)*

LIST OF TABLES

List of Tables *(continued)*

List of Tables *(continued)*

List of Tables *(continued)*

List of Tables *(continued)*

LIST OF ABBREVIATIONS

CSD	Cambridge structural database
DH	monoanion of dimethylglyoxime
dmbzim	5,6-dimethylbenzimidazole
ESR	electron spin resonance
En	ethylenediamine
FAB(MS)	fast atom bombardment mass spectrometry
HMBC	heteronuclear multiple bond correlation
HMQC	^{1}H-detected hetronuclear multiquantum correlation
HPLC	high pressure liquid chromatography
MPA	Mulliken population analysis
NMR	nuclear magnetic resonance
PM3	a reparameterisation of the Austin model 1 force field
ROESY	rotating frame Overhauser effect spectroscopy
Saloph	salicyclaldehydephenylenediimine
TOCSY	total correlation spectroscopy
UV-vis	ultraviolet-visible
ZINDO/1	Zerner's non-spectroscopic version of the intermediate neglect of differential overlap

Buffers

CAPS	3-[cyclohexylamino]-1-propanesulfonic acid[†]
CHES	2-[*N*-cyclohexylamino]-ethanesulfonic acid
HEPES	*N*-[2-Hydroxyethyl]piperazine-N´[2ethanesulfonic acid]
MES	2-[*N*-Morpholino]ethanesulfonic acid
MOPS	3-[*N*-Morpholino]propanesulfonic acid
Tris	Tris(hydroxymethyl)aminomethane

[†] Sulfur rather than sulphur is the IUPAC-recommended spelling and will be used throughout this thesis for related words.

Symbols

A	Absorbance
j	coupling constant
K_a	ionisation constant
K_L	acid dissociation constant for L
K_{OS}	Equilibrium constant for formation of the outer-sphere complex between L and cobalamin under investigation
K_{obs}	experimentally determined equilibrium constant for coordination of L by the cobalamin under investigation
k_{cor}	observed rate constant corrected for pH effects
k_{obs}	experimentally determined rate constant for the substitution of H_2O by L in the cobalamins
k_{sat}	saturation rate constant